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(54) Title: MONOCLONAL ANTIBODIES TO RENAL CELL CARCINOMA

(57) Abstract

Antigens associated with renal cell carcinoma (RCC) are described. The antigens are present on renal carcinoma cells and to varying extent on other benign and malignant tumors and normal cells. Compositions containing antibody against an antigen or mixture of antibody and their use in theraphy and diagnosis of RCC are also described.

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# MONOCLONAL ANTIBODIES TO RENAL CELL CARCINOMA

## Background of the Invention

Since the introduction of the hybridoma technique for production of nonspecific antibody by Kohler and Milstein, Nature 256: 495-497 (1975), efforts have been made to identify antigens that are present only on malignant tumors and to isolate monoclonal antibodies (Mabs) having a high specificity for these tumor antigens. See e.g., Colcher et al. Proc. Nat'l. Acad. Sci., 78: 3199-3203 (1981). Wright et al., Cancer Res. 43, 5509-5516 (1983); Wilson et al., Int. J. Cancer 28; 293-300 (1981) and Magnani et al., Cancer Res. 43:5489-5492. Mabs recognizing such antigens can be useful in reagents in diagnostic tumor pathology, and in serum tests for tumor associated or tumor specific antigens. Radiolabeled Mabs specific for these antigens can also be useful for detection, localization and treatment of these tumors in vivo.

Renal cell carcinoma (RCC) is a cancer which is particularly difficult to diagnose. See Oberling et al. Nature 186, 402-403 (1960) and Holthafer et al., Lab. Invest. 49, 319-326 (1983). Mabs specific to antigens which are pre-dominantly present only on RCC tumor cells would be extremely useful in the diagnosis and treatment of this disease.

Summary of the Invention

This invention pertains to several new antigens associated with renal cell carcinoma (RCC) and to monoclonal antibodies reactive with the antigens. The invention also pertains to methods of diagnosis and method of treatment of RCC and diagnostic and therapeutic compositions useful in these methods.

The RCC associated antigens of this invention are designated G 250, RC 38, RC 3, RC 69 and RC 154 and are characterized by distinct patterns of tissue distribution as elaborated below.

Antigen (Ag) G 250 is an antigen present on RCC and absent on normal adult fetal kidney tissue. Ag G 250 is absent from all other normal adult tissue (except for the epithelium of the bile ductand stomach) and most malignancies.

Antigen RC 38 is an antigen which is present on primary and metastatic RCC tumors. The RC 38 antibody reacted with 46 out of 47 primary RCC tested and with 8 out of 13 RCC metastases. No reaction was seen with 179 tumors of various origin. RC 38 is also present on the mucous cells of the stomach, on the crypts and villi of the jejunum, on the crypts of the colon, on the sinuses of the liver, on the acini of the sweat glands, and on the sinuses of the lymph node. It is also present on the glomerular visceral epithelium and the epithelial cells of the proximal tubules up to the thin descending part of Henle's loop on tissue sections of normal adult kidney. Further, RC 38 is present on the differentiating visceral glomerular epithelial cells at the capillary

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loop stage and on the most proximal part of the tubules connected to these regions of the fetal metanephros.

Antigen RC 3 is present on primary RCC and marginally present on metastic RCC. Antigen RC 69 is present on primary RCC but absent on metastic RCC. Both RC 3 and RC 69 are present on proximal tubules and the thick descending part of Henle's loop in tissue sections of normal adult kidneys. RC 3 is marginally present on the parietal epithelial cells of Bowman's capsule in the region adjoining the outgoing tubule. RC 69 is present on the fetal metanephros at the middle limb of the S-shaped stage. RC 3 and RC 69 were not present on any non-renal tissues which were tested.

Antigen RC 154 is present on primary RCC and absent on metastic RCC. RC 154 is marginally present on the proximal tubular epithelium and on the distal tubules and small collecting ducts of the normal adult kidney. It is present on the fetal metanephros, on the ducts of adult breast glands and on the follicles of the adult thyroid gland.

Antibodies reactive with the RCC antigens G 250, RC 38, RC 3, RC 69 and RC 154 can be used in the diagnosis and treatment of RCC.

## Brief Description of the Drawings

Figure 1 (Left) shows immunoperoxidase/(DAB) staining of RCC adjacent to uninvolved kidney tissue with Mab

G 250. (Magnification 64x.) Diffuse staining of RCC tumor cells (bottom), uninvolved kidney tissue negative.

Right: GAM_FITC staining of primary RCC with G 250. (Magnification 512x.) Diffuse, membranous staining of RCC tumor cells is visible.

Figure 2. shows immunoperoxidase/DAB staining of normal liver with G 250 (magnification 160x; Magnification inset 880x) Cytoplasmatic staining of bile canuliculi is visible. The cytoplasmatic aspect of the staining is visible in the inset.

Figure 3.

Fig 3a, b: Radioimmunoscintigraphy of an RCC tumor bearing mouse, 2 hours (fig 3a), and 20 hours after injection (fig 3b), 100,000 count images per figure. Fig 3c: Radioimmunoscintigraphy of a melanoma tumor bearing mouse, 20 hours after injection, 50,000 count images. Mice were given an intraveneous injection of 1.5 ug $^{99m}$ Tc labeled G 250. In figure 3a, b the RCC tumor as well as the liver are visible 2 and 20 hours after injection. In fig 3a the injection point in the tail is also visible. In the melanoma bearing mouse (fig 3c), only the liver is visible.

Figure 4 is a bar histogram indicating staining reaction of malignant tumors with G 250. (black:

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more than 50% of tumor cells stained; striped: more than 1% and less than 50% of tumor cells stained; dotted: less than 1% of tumor cells stained; white: no tumor cells stained; Ca = carcinoma). Numbers between brackets are total numbers of tumors tested. These represent mostly primary tumors. The metastases were: nine mammary tumors (one with less than 1% tumor cells positive, eight negative), four pulmonary tumors (all negative) and four colonic tumors (one with more than 50% positive tumor cells, onw with less than 1% tumor cells positive, two negative). Percentages in bars represent percentage of tumors with staining characteristics corresponding to bar color.

Detailed Description of the Invention

The antibodies of this invention react with antigens present on RCC. The antigens are designated G 250, RC 38, RC 3, RC 69 and RC 154. In brief, RC 38 reacts with 95% of primary and 67% of metastatic RCC and did not react with other tumors tested (but did react with some normal tissue). G 250 reacts with primary and metastatic RCC but not with normal tissue. RC 4 reacted with RCC and a wide spectrum of tumors.

G 250

The presence of Ag G 250 was determined by the staining reaction of the antigen with Mab G 250. G 250 is present in a high percentage of cells in most RCC tumors and is absent from the cells of

normal kidneys. With respect to the normal tissues tested G 250 is present on normal bile duct epithelium, mucous cells in the stomach and is marginally present on epithelial cells in the jejunum. No reaction was found with other adult tissues tested nor with any fetal tissue tested.

Expression of G 250 antigen was found in a few benign tumors and premalignant lesions including the two renal adenomas tested. The G 250 antigen appears to be absent from the normal adult kidney as evidenced by the data obtained in ELISA and immunohistochemistry. The G 250 appearance in renal adenoma and the general occurrence of this antigen in primary RCC suggest that induction of G 250 antigen synthesis is inherently related to tumor development, possibly due to a common initiating event such as activation of a cellular oncogene product. The G 250 determinant was also found in nonRCC tumors, mainly in carcinomas but occasionally also in sarcomas. In these cases the corresponding normal tissues were negative. G 250 antigen is expressed relatively frequently in colonic carcinomas.

The fact that G 250 stains a variety of other tumors, although with low incidence, makes it less suitable for establishing a differential diagnosis of RCC. The strong staining of cell membranes in the majority of RCC tumor cells in most RCC suggests that this Mab can be useful for tumor scintigraphy. In radioimmunoscintigraphic experiments, RCC tumors from

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a cell line with moderate expression of G 250 antigen (as estimated from the immunofluorescence data) were visualized. Clear tumor resolution was obtained with RCC tumors with a diameter ranging from 5-7mm without using subtraction techniques. Tumors were also visualized with labeled G 250 in tumor bearing kidneys ex vivo. These data and the fact that the G 250 antigen is present in only a few normal tissues suggests that antibodies specific to G 250 antigen are useful for RCC diagnosis.

The RC Series

Antigens RC 3, RC 69, RC 154 and RC 38 were also discovered on the cells of renal cell carcinoma tumors. These antigens were discovered by their reaction with a series of monoclonal antibodies. RC 3 antigen reacted with Mab RC 3, RC 69 antigen with Mab RC 69, RC 154 antigen with Mab RC 154 and RC 38 antigen with Mab RC 38. These monoclonal antibodies and the cell lines which produced them are specific embodiments of the monoclonal antibodies and cell lines of this invention.

By their reactions with their respective Mabs, the expression of the antigens RC 3, RC 69, RC 154 and RC 38 was found to be variable both with respect to numbers of antigen expressed and to the percentage of antigen-positive RCC tumor cells. The combinations of antigen expression RC 3+/RC 69+/RC 154+ and RC 3+/RC 69+/RC 154- were frequently observed in primary RCC whereas no tumors were found to have a

combination of antigen expression RC 3-/R C69+/RC 154+ or RC 3-/RC 69-/154+.

RC 69 antigen and RC 154 antigen were not found in RCC metastases while RC 3 was found on only 1 out of 10 of the metastases tested.

The data indicates that RCC cells with metastasizing capacity have lost the antigens corresponding to RC 3, RC 69 and RC 154.

Of the renal adenomas only one expressed RC 38 antigen, while six others did not express RC 38 antigen. RC 3, RC 69 and RC 154 antigen were not expressed in the two renal adenomas tested. The surrounding proximal tubular epithelium was clearly stained in all sections. Renal adenomas are often considered precursor lesions of RCC and the main distinguishing point between adenoma and carcinoma is considered to be the size of the lesion, diameter 3 cm constituting the borderline between these lesions See Bennington, "Histopathology of Renal Tumors" in: Renal Adenocarcinoma (UICC Technical Reporting Series No. 49) Geneva, UICC, 1980, Eds. Sufrin, G. and Buckley, A. Our limited data on small renal adenomas only suggest that a subset of these adenomas do not represent a transitional stage from normal proximal tubules to RCC as proximal tubular epithelium and primary RCC always express RC 38 antigen. The data are compatible with an origin from distal tubular epithelium.

RC 38 antigen is not present on tumor cells of breast tumors, carcinomas of the gastrointestinal

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tract or angiosarcomas. In most tumors, RC 38 antigen is present on the endothelium of small vessels whereas it is absent in capillary endothelium of normal tissues except in the liver and lymph nodes. Therefore RC 38 might be useful in studies on angiogenesis or vascularisation in human tumors.

Combining the data of tables 4 and 8 it is seen that tumor cells of 46 out of 47 primary RCC and 8 out of 13 metastatic RCC were stained with RC 38. Also, RC 38 did not stain tumor cells of a wide variety of other tumors that included 12 clear cell tumors of different origins. These data indicate that RC 38 is useful for diagnostic purposes.

Immunoassay for RCC antigen

Antibodies against the enumerated RCC antigens can be used to detect RCC antigen in samples of bodily fluids (e.g. serum or plasma). For example, serological tests for circulating antigen may have diagnostic or prognostic value. Immunoassays for detection of RCC antigens can be performed in any of the standard formats such as competitive or immunometric formats.

Immunohistochemical Staining

Human tissue specimens (e.g. biopsy samples) can be tested for the presence of the RCC antigens by immunohistochemical techniques such as immunoperoxidase staining. As an alternative to immunoperoxidase staining immunofluorescent techniques can be used to

examine human tissue specimens with the anti-RCC antibodies. In a typical protocol, slides containing cryostat section of frozen, unfixed tissue biopsy samples or cytological smears are air dried and incubated with the anti-RCC antibody in a humidified chamber at room temperature. The slides are then layered with a preparation of antibody against the anti-RCC antibody. For example, if a murine anti-RCC antibody is used the second antibody can be an anti-mouse antibody. The second antibody is labeled with a fluorescent compound. The staining pattern and intensities within the sample are then determined by fluorescent light microscopy.

Immunoscintigraphy

An immunoscintigraphic image of RCC in vivo can be obtained by administering to a person suspected of having RCC, labeled antibody (or a mixture labeled of antibodies) against an RCC antigen and allowing sufficient time for the antibody to accumulate at the tumor site(s). The signal generated by the labeled antibody is then detected by an appropriate detecting device and the detected signal is converted to an image of the tumor. The constructed image can be used to localize and to assess the size of the tumor in vivo.

As immunoscintigraphic agents, antibodies against antigens G250 and RC 38 are preferred. These

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antigens are expressed by primary and metastatic RCC and thus can abe used to localize primary and disseminated tumor. As noted, mixtures of antibody (i.e., "cocktails") can also be used. For example, a mixture of the antibody G 250 and RC 38 can provide an imaging composition having the advantages of the properties of both antibodies.

For radioimmunoscintigraphy in humans, radioisotopically labeled intact antibody or antigen-binding fragments of anti-RCC antibody such as the monovalent Fab' fragment or the divalent $F(ab')_2$ fragment may be used. The advantages of using antibody fragments in tumor radioimaging techniques are described by Goldenberg, D.M. and Deland, F.H. (1982) J. Biol. Response Modifiers 1, 121-136 and by Goldenberg in U.S. Patent 4,331,647.

The preferred label for immunoscintigraphy is a gamma-emitting radioisotope which can be detected with a conventional photoscanning device (such as a gamma camera). Examples of gamma emitting radioisotopes conventionally employed in in vivo tumor radioimaging techniques including $^{123}$Iodine, $^{125}$Iodine, $^{131}$ Iodine, $^{99m}$Technetium or $^{111}$Indium. A variety of methods exist for attaching the radioisotopes to proteins either directly or via a chelating agent such as diethylene triamine pentacetic acid (DTPA); any of these may be used to label the antibody or antibody fragment. For instance, the

antibody may be labeled with Na[$^{125}$I] by the chloramine-T method. See Hunter, W. M. and Greenwood, F. C. (1962), Nature 194, 495. Antibody may be directly labeled with $^{99m}$ Technetium by the technique of Crockford et al., U.S. Patent No. 4,424,200, or it may be attached via a DTPA chelate as described by Hnatowich, U.S. Patent 4,479,930. In general, the antibody or antibody fragment is labeled to an appropriate specific activity (generally at least about 5 uCi/ug protein).

The immunoscintigraphic composition is injected into the patient intravenously, intra-arterially or intraperitoneally. The amount of radioactivity injected should be sufficient for detection by a standard gamma camera after the labeled antibody has distributed through the tissues of the body.

The anti-RCC antibodies of this invention may be provided in kits for radioimmunoscintigraphy in humans. Preferably, such a kit includes either antibody the monovalent fragment Fab', the bivalent fragment $F(ab')_2$ or a cocktail of antibodies or antibody fragments (e.g. G250 and RC 38). In general, the labeling procedure will be prepared by the clinician. The antibodies or fragments can be provided with a preattached chelator (e.g. DTPA) for labeling via the chelator. The labeled antibody is prepared for injection in a physiologically acceptable vehicle.

Imaging based on the detection of nuclear magnetic resonance (NMR) properties of tissues labeled with paramegnetic substance (such as compounds containing manganese) can also be employed. Monoclonal antibodies may be used to deliver the

paramagnetic substance to the RCC site and allow detection of tumor masses by NMR imaging.

Therapeutics

The RCC-associated antigens of this invention also provide a basis for therapy of RCC. Antibody targeted to RCC antigens can be administered in therapeutically effective (anti-tumor) amounts to patients afflicted with RCC. The antibody can be given alone or as a carrier of an anti-tumor drug. Among the various antiproliferative, antineoplastic or cytotoxic agents that may be linked to the anti-RCC antibody are antimetabolites, such as the anti-folate, methotrexate, or the purine or pyrimidine analogs mercaptopurine and fluorouracil. Others include antibiotics, lectins such as ricin and abrin, toxins such as the subunit of diphtheria toxin, radionuclides such as $^{211}$Astatine and $^{131}$Iodine, radiosensitizers such as misanidazole or neutron sensitizers such as boroncontaining organics. These agents may be attached to the antibody by conventional techniques such as glutaraldehyde cross-linking.

Targeting Cytotoxic Cells

Antibodies against the RCC antigens can be used to target cytotoxic cells (e.g. human T cells, monocytes or NK cells). Cytotoxic cells can be attached to RCC via Fc receptors on the cells (which bind the Fc portion of an anti-RCC antibody) or via a

bridging antibody of dual specificty (i.e. an antibody specific for the cytotoxic cell and for RCC).

Dual or bi-specific antibodyes for targeting RCC can be produced by fusing an anti-RCC producing cells with a cell producing antibody against the cytotoxic cell to be targeted. For example, a cell formed by fusion of a hybridoma producing anti-RCC antibody and a hybridoma producing anti-cytotoxic cell antibody (quadroma) will produce hybrid antibody having specificity of the antibodies produced by the parents. See, e.g. Immunol. Rev. 1979; Cold Spring Harbor Symposium Quant. Biol. 1977: 41, 793. Thus, a hybridoma producing an anti-T3 antibody can be fused with hybridoma producing antibody against the RCC antigen (preferably RC 38 or G250) to yield a cell line which produces T3/RCC bispecific antibody for targeting cytotoxic T cells to RCC. Bispecific antibodies can also be produced as heteroantibodies by chemically coupling two antibodies of desired specificity.

The cytotoxic cell can be targeted by allowing the bispecific antibody to bind the cell. After targeting, the cells can be adminstered to the patient. Therapy with targeted cells can be used as an adjunct to surgical therapy, radiation therapy, or chemotherapy of RCC.

Class Switch Variants

Monoclonal antibodies against the RCC antigens G 250, RC 38, RC 3, RC 69 and RC 154 of different

immunoglobulin classes can be prepared by techniques for class switching of hybridoma antibodies. See, e.g. J. Immunol., 1982:128, 1271; J. Immunol. 1983:131 877; PNAS 1980 77, 2909 and PNAS 1985: 82,8653. Antibodies of the various G subclasses (1, 2a, 2b and 3) can be produced which bind to different Fc receptors on effector cells. Antibody of the A, M, D and E classes can also be produced. IgE variants may interact with mast cells to provide an allergic effect at the tumor site.

The invention is illustrated further by the following examples.

EXAMPLE 1

Production of the G 250 Hybridoma Cell Line

An RBF mouse was immunized 5 times (with four week intervals) with cell homogenates from primary RCC lesions obtained from 4 different patients. Cell homogenates were diluted 1:1 with Freund's incomplete adjuvant (Sigma, St. Louis, USA). Three days after the last immunization the mouse was sacrificed and the spleen cells were isolated. These were fused with Sp2/0 myeloma cells essentially according to Kohler and Milstein, Nature, 256, 495-497 (1975), and cultured in soft agar. After 10 days of growth, the agar was overlaid with nitrocellulose filters, Sharon et al., Proc. Natl. Acad. Sci., 76, 1420-1424 (1979), coated with a mixture of 4 RCC cell homogenates not used for the immunization procedure. A second filter coated with 0.5% gelatin was also applied to identify clones producing antibody binding to irrelevant

antigens. After overnight incubation, the filters were removed and incubated with rabbit antimouse Ig conjugated to horseradish peroxidase (RAM-HPO), washed and stained with 0.05% 3-3' diaminobenzidine (DAB) and 0.03% $H_2O_2$. Colonies producing antibodies giving spots on the RCC coated filter only were picked and grown in suspension. Tissue culture medium from these clones was tested on cryostat sections of RCC lesions and normal kidney. Clones reacting with RCC and not with normal kidney tissue were subcloned and tested on other normal tissues.

## EXAMPLE 2

## Reactivity of G 250

## Tissue Samples, Staining of Cryostat Sections and Scoring Procedure

Tissue samples taken from surgical specimens, autopsy and abortions were snap-frozen and stored at -70°C until used. For RCC, at least two tissue blocks obtained fron non adjacent parts, were tested.

Indirect immunoperoxidase staining of air-dried and aceton fixed cryostat sections was done with hybridoma culture fluid essentially according to van Muijen et al. Am. J. Pathol., 114 9-17 (1984). RAM-HPO was used as a second antibody and DAB/$H_2O_2$ as substrates. Sections were counterstained with hematoxylin. For immunofluorescent staining of RCC sections, undiluted hybridoma culture fluid was applied as a first step and goat antimouse Ig coupled to FITC (GAM-FITC/ Nordic, Tilburg, The Netherlands)

as a second step. Sections were mounted and examined in a Leitz Orthoplan immunofluorescence microscope with Ploeaopak illuminator.

Cryostat sections of normal tissues were scored as negative when not a single cell was stained. In cryostat sections of benign and malignant tumors, percentages of cells stained per $cm^2$ were estimated visually. Four categories were arbitrarily distinguished. These were: negative, less than 1%, between 1% and 50%, and more than 50% of tumor cells stained.

EXAMPLE 3

Reactivity of Mab G 250-Staining of Cells

RCC cell lines SK-RC-1, SK-RC-6 and SK-RC-7 were obtained from Dr. L. J. Old, Memorial Sloane-Kettering Institute, New York, N. Y.

Single cell suspensions from fresh RCC specimen were obtained by collagenase treatment (Sigma, St. Louis, USA).

Unfixed or acetone fixed cells grown on glass were examined for the presence of G 250 antigen by immunofluorescence. Undiluted culture medium was used as first step and GAM-FITC as second antibody. The cells were examined in a Leitz Orthoplan immunofluorescence microscope with Ploemopak.

RESULTS OF EXAMPLES 1-3

Using the methods described above, a clone of hybridoma cells designated G 250 was isolated that produced antibody of the IgG1 subclass reacting with

cryostat but not with formalin-fixed and paraffin embedded tissue sections of RCC. G 250 did not stain any structure in cryostat sections of normal kidneys ( 50 cases), rejected transplant kidneys (8 cases) or kidney biopsies from 5 SLE patients. Increasing the antibody concentration 40x and enhancing the staining reaction with imidazole (Straus, J. Histochem. Cytochem., 30, 491-493 (1980)), also did not lead to staining of normal kidney structures.

The staining reaction of RCC cryostat sections could be abolished by preabsorption with sup 2 of RCC but not with proteinase K (Sigma, St. Louis, USA) digested sup 2 RCC fractions. Pretreatment of RCC tissue sections with 20mM NaIO did not reduce the staining reaction. The sensitivity to proteinase K suggests that G 250 recognizes a protein. The antigen present in crude RCC homogenates or in sup 2 could not be characterized in immunoblotting experiments done essentially according to Towbin et al., Proc. Natl. Acad. Sci., 76, 4350-4354, (1980), and could not be purified by affinity chromatography on Sepharose-G 250 columns.

Several other tumor types expressed the antigen detected by Mab G 250. However, the fraction of tumors stained, the percentages of G 250 positive tumor cells and the intensity of staining were generallly much lower. The staining of positive non RCC tumor cells always appeared to be cytoplasmatic.

In addition to the above-mentioned tumors, small numbers of a few other tumor types were tested (See Table 2). These were two Wilm's tumors (both negative), one prostatic carcinoma (negative), five adrenal cortical carcinomas (4 negative, one with more than 50% tumor cells positive), five gastric carcinomas (all negative), two liver cell carcinomas (both negative) and one carcinoma of the renal pelvis (more than 50% tumor cells positive).

Several benign tumors and premalignant lesions were tested with Mab G 250 (see Table 2). In two renal adenomas (diameter 10mm and 20mm) all cell membranes were stained. Four cases of epitheliosis of the breast were negative, and 11 cases of colonic adenomas showed one case with less than 1%, and two cases with less than 50% of positive cells respectively. Of 6 mixed tumors of the salivary gland four were negative, and the two others showed less than 50% of positive cells. One pheochromocytoma was tested and had more than 50% positive cells.

Staining of Viable Cells and Cell Lines

Using G 250 antibody, staining of cell membranes was observed in unfixed single cell suspensions obtained after collagenase treatment of fresh RCC specimen. Also, membranous staining of RCC cellines SK-RC-1 and SK-RC-7 was observed while no reaction was seen with SK-RC-6. No fluorescence was seen on human embryonic kidney cell lines subcultured 6 times or on HEK 293, an embryonic human kidney cell line

transformed with adenovirus. The monkey renal cell lines BSC-1 and CV-1 were both negative.

EXAMPLE 4

Enzyme Linked Immunosorbent Assay Using G 250

One gram of RCC or normal kidney tissue was homogenized in 3 ml of 10 mM Tris-HCl, pH 7.4, in a Potter Elvehjem apparatus (5 strokes at 1500 rpm). After centrifugation (10 min 10,000g), the supernatant was stored at -70°C (= sup 1) and the pellet was resuspended in 3 ml of 10mM Tris-HCl, pH 7.4, containing 0.1% Triton X-100. After 10 min at 0°C this suspension was centrifuged for 10 min at 10,000g. The supernatant was dialyzed overnight against 10 mM Tris-HCl, pH 7.4 (= sup 2).

Presence of relevant antigen in the respective homogenates was tested in a checkerboard assay. In short, wells in microtiter plates (Sterilin Limited, Teddington, U. K.) were filled with 100 ul of dilutions of sup 1 and sup 2 in 0.1 M $Na_2CO_3$, pH 9.5. The contents were allowed to evaporate overnight at 37°C. After blocking remaining binding sites with 3% ovalbumine in phosphate buffered saline, the wells were incubated for 2 hours at 37°C with 100 ul of G 250 culture medium. After washing and incubating with RAM-HPO, the plates were washed again and were developed with 200 ul of 0-diphenylamine and $H_2O_2$. The reactions were stopped by adding 50 ul 2.5 M $H_2SO_4$ and optical density readings were taken at 492 nM. As controls, Sp2/0 media, not containing

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antibody were used with and without RAM-HPO as second antibody.

Using normal kidney and RCC homogenates in ELISA as coating material, G 250 antigen was present only in the sup 2 fraction of RCC. The OD492 readings of wells coated with normal kidney extracts and incubated with G 250 culture medium did not exceed the values of Sp2/0 or RAM-HPO incubated wells. The OD492 readings on kidney extract coated wells were in the range of 0.01-0.03, whereas the OD492 readings on RCC sup 2 coated wells were in the range of from 1.0-2.0.

## EXAMPLE 5

### Radioimmunoscintigraphy Using Mab G 250

G 250 antibody was purified from ascites fluid prepared in F1 (RBFxBalb/C) mice by chromatography on DEAE-AFFi-Gel Blue (Bio-Rad Laboratories, Richmond, USA).

Purified G 250 IgG1 was labeled with $^{99m}$Technetium. Immunological activity of the $^{99m}$Tc labeled G 250 preparation was evaluated in ELISA-tests.

Human RCC-xenografts were established by seeding $10^6$ SK-RC-1 subcutanously in nude mice. Tumor bearing mice were used four weeks after seeding of the tumor cells. As a control, a nude mouse bearing a tumor derived from human melanoma cell line BRO (Lockshin et al., Cancer Res. 45, 345-350, 1985) was used.

Scintigraphy was performed using a Gamma Toshiba GCA 40A gammacamera, connected with an MDS A2 computer system. 100,000 count images were acquired over a 5 min period 2 hours after injection and over a 30-40 min period 20 hours after injection. Background subtraction techniques were not utilized.

Two mice bearing RCC tumors with diameters of 5 and 7 mm and one mouse bearing a melanoma tumor with a diameter of 6 mm were given intravenous injections of 1.5 ug $^{99m}$Tc labeled G 250 antibody with a specific activity of 132 uC/ug protein. The immunological activities of the $^{99m}$Tc labeled and unlabeled G 250 peparations were evaluated in ELISA and were similar. After two hours, a scintigraphic distinction of the RCC tumor could be made (Fig. 3a). The xenografts were distinctly visible 20 hours after injection as was the region of the liver (Fig. 3b). After 20 hours, 7% of the total body counts were accumulated in the RCC tumors. In the melanoma bearing mouse, no tumor was visible after 2 or 20 hours after injection (Fig. 3c). The smallest tumor visualized weighed 60 mg and measured 5x5x4 mm.

## EXAMPLE 6
## Preparation of Hybridoma Cell Lines for RC 38, RC 3, RC69 and RC 154

Source of Tissues

Tumor tissue samples, excluding necrotic and haemorrhagic areas were taken from surgical specimens

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of various malignancies. In three cases of RCC, both primary and metastatic tumors were obtained at surgery. In two cases of RCC, autopsy material was used. In these two cases, primary tumor and corresponding metastases were obtained. From each RCC several non adjacent tumor samples were taken. Renal adenomas were obtained at autopsy.

Normal tissues used for specificity tests of Mabs from autopsies performed within a few hours after death or from uninvolved parts of surgical specimens. These included kidney, ureter, bladder, stomach, jejunum, colon, testis, cervix, pancreas, prostate, lung, liver, breast, skeletal muscle, brain, lymph node, uterus, thyroid gland, adrenal gland and skin.

All Mabs described here were tested on at least three different tissue sections of the aforementioned normal tissues obtained from different patients.

Fetal kidney tissues of 11-, 13-, 14-, 15-, 18, and 20 week gestation as estimated from bodylength measurements were obtained from abortions.

All tissues were snap-frozen and stored at -70°C until used.

Preparation of Cell Homogenates

Cell homogenates were prepared from adult renal cortex or medulla and from RCC. Tissue was homogenized with a Potter Elvehjem homogenizer (5 strokes at 1500 rpm) in three volumes of phosphate buffered saline pH 7.4 (PBS). After centrifugation for 10

min. at 1000g, the supernatant was used for immunization purposes or for coating nitrocellulose filters.

Immunization

Balb/c mice were used for immunization purposes. Each animal was immunized at least three times with homogenates prepared as described above. For the immunization with RCC, homogenates from three different patients were used. For the first injection the tissue homogenate was mixed with an equal volume of Freunds complete adjuvant (Sigma, St. Louis, USA). Each animal was injected with 0.5 ml of the mixture of tissue homogenate and Freunds adjuvant. Booster injections with Freunds incomplete adjuvant (1:1, Sigma, St. Louis, USA) were given at two week intervals. Three days after the last injection the mice were killed and the spleen cells were used for fusion.

Fusion of Spleen Cells with Sp2/0 and Detection of Hybridomas Producing Relevant Antibody

The spleen cells of immunized mice were fused with Sp2/0 cells essentially according to Kohler and Milstein. After fusion, the cells were plated into 20 petridishes, diameter 5 cm, in soft agar (0.4%) and incubated for 10 days at 37°C in a $CO_2$ incubator. Then the agar was overlaid with nitrocellulose

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filters saturated with a cell homogenate corresponding to the homogenate used for immunization and a second filter, saturated with 0.75% gelatin dissolved in Hanks balanced salt solution or saturated with a homogenate made from a normal adult human liver, as a first screen to discrimate between kidney relevant and irrelevant antibody producing colonies. These filters were prepared as follows: Cell homogenates were diluted 20 fold in Hanks balanced salt solution and sonified. The filters were soaked in Hanks balanced salt solution and put on a sintered glass funnel of diameter 47 mm. Then 10 ml of the cell homogenate was sucked through and the filters were air-dried and sterilized by UV light (30 Watt Philips TUV at 90 cm, 2 times 20 minutes each side). To reduce toxicity of the filters, the filters were washed in sterile water. Thereafter the filters were soaked in HAT-medium containing fetal calf serum to block remaining protein binding sites. After overnight incubation on the soft agar, the filters were removed and incubated for one hour with rabbit-anti-mouse Ig conjugated to horseradish peroxidase. After extensive washing in 10mM Tris-HCl, pH 7.4, containing 0.02% sodium-dodecyl-sulphate and 0.5% sarcosyl NL 30 (Ciba-Geigy B.V., Arnhem, The Netherlands), the filters were developed with 0.05% di-amino-benzidine and 0.03% $H_2O_2$ in 50 mM Tris-HCl, pH 7.4. The procedure followed was adapted from Sharon et al, Pro Natl Acad Sci, 76 1420-1424 (1979).

Colonies producing antibodies that reacted with the filters coated with the relevant cell homogenate

but not with the filters coated with liver cell homogenate or gelatine were picked and grown in suspension in microliter plates. Undiluted culture media of these cells were tested on cryostat sections of several tissues. Colonies producing antibodies positive on adult renal tissue sections or RCC sections and negative on liver and lung tissue sections were subcloned and further analyzed on frozen sections of other tissues.

## EXAMPLE 7

## Staining and Scoring Procedure of Mab RC 38, RC 3, RC 69 and RC 154

To test the specificity of the Mabs and to identify the structures stained, indirect immunoperoxidase staining was performed on frozen sections of various normal tissues, fetal kidneys and tumors as described by van Muijen et al., Am. J. Path., 114, 9-17 (1984), with the exception that 3,3'-di-aminobenzidine was used as substrate. Sections were counterstained with hematoxylin.

Sections of normal tissues were scored negative when not a single cell was stained. Tumors were scored as negative when no tumor cells were stained, e.g. tumor sections in which only blood vessels were stained were considered to be negative.

The subsite of the nephron stained by the Mabs was established by using generally accepted morphologic criteria such as presence of brush-border and width of tubule lumen. The position of the tissue

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section in the kidney was also taken into consideration. Rabbit-anti-human Tamm Horsfall protein (RAH-THP) was used to identify the ascending limb of Henle's loop and the distal convoluted tubule. Double immunofluorescence staining was performed to identify any overlap of THP containing cells and the cells stained with the Mabs.

EXAMPLE 8

Test Set Of Poorly Differentiated Malignant Tumors For Which The Diagnosis Of RCC Had Been Considered

To confirm the diagnostic potential of Mab RC 38, a test set of diagnostically difficult tumors was selected. Tumors of this test set included three cytological aspirates which present additional diagnostic difficulties, and poorly differentiated malignant tumors with a histological appearance and clinical presentation that mimicked RCC. The possibility of RCC had therefore been considered by the pathologist in the differential diagnosis. The histological appearance of these cases included adenocarcinoma with clear cell, cribriform or acinar patterns, undifferentiated large-cell malignant tumors and spindle cell malignant tumors. None of these cases were included in the series of tumors used to test the specificity of the Mabs.

Tumors of the test set were stained and scored by E. O. without knowledge of the final diagnosis.

RESULTS OF EXAMPLES 6-8

Origin and Subclass of Monoclonal Antibodies

Mab RC 38, subclass IgG1, was derived from fusion of the spleen cells of a mouse immunized with RCC homogenates. RC 3, subclass IgG1, was developed with spleen cells from a mouse immunized with normal adult renal cortex homogenates. RC 69 and RC 154 subclass IgG2b and IgG2a respectively were derived from fusion of spleen cells of a mouse immunized.

All Mabs are only applicable on cryostat sections. When tested on formalin-fixed tissue, no staining reaction was observed.

Staining of Normal Human and Fetal Tissue Sections

RC 38

In tissue sections of adult kidney RC 38 stained the glomerular visceral epithelium and the epithelial cells of the proximal tubules up to the thin descending part of Henle's loop. Staining of the proximal tubular cells was mainly localized within the cytoplasm. No staining of the THP containing cells--present in the distal tubules--was seen as indicated by double immunofluorescence.

In sections of the fetal metanephros, RC 38 stained differentiating visceral glomerular epithelial cells at the capillary loop stage and the most proximal part of the tubules connected to these regions.

In non renal tissue sections, RC 38 stained the surface of the epithelium of the jejunum and colon, the mucous cells of the faveolar and glandular layer

of the stomach mucosa and acini of sweat glands. In addition sinusoidal lining cells in the liver and lymph nodes were stained. Other tissues tested were negative (Table 3).

RC 3 and RC 69

In tissue sections of adult kidney RC 3 and RC 69 stained the proximal tubules up to the descending part of Henle's loop. No staining of more distal parts of the nephron was observed. The staining of proximal tubular cells was mainly associated with the brush-border. RC 3 faintly stained the parietal epithelial cells of Bowman's capsule in the region adjoining the outgoing tubule.

In sections of the fetal metanephros the middle limb of the S-shaped stage, the part that will eventually develop into the proximal tubule was stained with RC 69. Developing proximal tubules and differentiating parietal epithelium were heavily stained with both RC 3 and RC 69.

All non renal tissues tested were negative with RC 3 or RC 69 (Table 5).

RC 154

In adult kidney tissue sections, a weak baso-lateral staining of the proximal tubular epithelium was observed with RC 154 in addition to intense cytoplasmatic basolateral staining of the distal tubules and small collecting ducts. In double immunofluorescence, the distal tubular epithelium was

positive with both RC 154 and RAH-THP, whereas the proximal tubular epithelium was weakly positive with RC 154 only.

In the fetal metanephros essentially the same distribution was seen: More intense staining of the distal parts of the differentiating nephron as compared to the more proximal parts. Nephrons were stained after the development of the capillary loop stage.

In non renal tissue sections, weak staining of ducts in breast gland and follicles in thyroid gland tissues sections was observed. Other tissues tested were negative (table 3).

Staining of tumors

The staining results on RCC and other tumors from the urogenital tract are summarized in table 4. RC 38 stained 95% of primary and 60% of metastatic RCC. RC 69 and RC 154 reacted with 70% and 40% of primary RCC, respectively. RC 69 and RC 154 did not stain the sections of metastatic RCC tested.

Other tumors of the urogenital tract and various tumors originating outside the urogenital tract were not stained by any of the Mabs (tables 4, 5). These tumors included clear cell type tumors of the testis (6x), lung (2x), ovary (3x) and soft tissue (1x).

With RC 38 staining of endothelial cells of blood capillaries was often observed in all tumors.

In table 6 the percentages of tumor cells stained with the Mabs are indicated. The staining of

RCC was heterogeneous for all four Mabs and ranged from a few positive tumor cells to diffuse staining of tumor sections. With RC 38 a glomerulus, the proximal tubules and tumor cells are stained. With RC 3 and RC 69 (data not shown) the proximal tubules and tumor cells are stained.

The percentages of tumor cells stained were quite similar in all pieces of one tumor studied except for two tumors where RC 3 failed to stain one tissue block and a few positive tumor cells were observed in another tissue block. In table 4 the percentages of tumor cells stained with the Mabs are indicated. These percentages were estimated visually. In tissue sections of three RCC only 50-100 positive tumor cells per $cm^2$ of tumor section were seen with one or two Mabs corresponding to less than 1% of cells positive (see table 6) while higher percentages of tumor cells were stained with the other Mabs tested.

In metastatic RCC stained with RC 38 the same heterogeneity was seen as in primary RCC.

In primary RCC various combinations of the antigens recognized by RC 3, RC 69 and RC 154 are expressed as shown in table 7. 27 out of 41 primary RCC tested with these three Mabs stained with two or more of the Mabs, 7 with none. Note that some of the possible combinations of antigen expression were not observed.

In 5 cases primary RCC as well as a corresponding metastatic lesion was available. RC 3 and RC 69

stained two and RC 154 one of these primary RCC and none of the metastatic lesions, while RC 38 stained all five primary RCC and three metastases.

Staining of test set tumors

Four of the tumors (cases 1,2,3 and 4) in the test set of 18 tumors were stained with RC 38 antibody (table 8). In these 4 cases the ultimate diagnosis based on additional clinicopathological findings was RCC. Two other tumors which were compatible with RCC on histological and clinical grounds, were negative with RC 38. All other 12 tumors which were negative for RC 38 were eventually found to be non-renal on the basis of clinical or other histochemical studies.

Staining of renal adenomas

In one adenoma, diameter 10 mm, a few cells were stained with RC 38 while RC 3, RC 3 and RC 154 failed to stain any cell. In another adenoma, diameter 4 mm, no cells were stained by any of the Mabs. Five other adenomas -four from one patient- ranging in size from 2-4 mm were tested with RC 38 only and were found to be negative.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

EXAMPLE 9 Radioimmunoscintigraphy and Biodistribution in Nude Mice

Antibodies RC 38 and G 250 were used as radioimmunoscintigraphic (RIS) agents in nude mice bearing subcutaneous RCC and/or melanoma. Studies were done using $^{99m}Tc$, $^{125}I$ and $^{111}In$-labeled intact immunoglobulins (Ig) and $^{99m}Tc$-labeled F(ab')2 fragments. Various times after injection mice were imaged and sacrificed to study biodistribution. Both antibodies showed tumor specific localization in the RCC xenograft. No accumulation of an irrelvant antibody in the RCC xenograft or RC 38 and G 250 antibody in the melanoma xenograft was observed. RCC could be clearly seen from 1,5-4 hours post injection. Biodistribution indices (tumor:tissue ratios) showed that maximum levels in the tumor were reached about 24 hours after administration of the antibody. With all three radionuclides all organs had tumor:tissue ratios greater than 10 except the liver, kidneys and spleen with $^{99m}Tc$-label. Visualization of the tumor was possible with F(ab')2 fragments of both antibodies. Seven days after administration of the $^{111}In$-labeled Mabs the biodistribution indices for liver, spleen and kidneys had markedly improved resulting in excellent scans with very low background. In conclusion our studies indicate that RC 38 and G 250 are useful RIS agents for the detection of RCC.

EXAMPLE 10

Immunoscintigraphy Of Tumor Bearing Human Kidney

Ex vivo experiments were performed with two tumor bearing kidneys that were perfused with $^{99m}Tc$ labeled G 250. The first tumor slowly accumulated $^{99m}Tc$ G250 antibody. Slow accumulation was not unexpected as first images showed that the blood supply of the tumor was poor as compared to the normal kidney tissue. No accumulation occured in normal kidney tissue.

An image was obtained 2 hours after the first administration of $^{99m}Tc$ labeled G 250 antibody. At that time the tumor:normal kidney ratio was 1:1,4. A final image was obtained after washing for 10 hours with perfusion fluid not containing radiolabeled G 250 antibody. The tumor:kidney ration was 1,8:1. After examination by the pathologist it appeared that this kidney tumor was not an RCC but an oncocytoma of the kidney. In second tumor, fast accumulation of radiolabel was observed, whereas no accumulation of radiolabel was observed in the normal kidney tissue. An image was obtained 16 hours after the first administration of the labeled antibody. At that time the tumor:kidney ration was 4:1. After washing for 7 hours with fresh Collins fluid to remove unbound G250 antibody, this ratio increased to 9:1. After examination by the pathologist all hot spots visible coincided with tumor tissue. A hot spot on the right side of the kidney coincided with tumor tissue

protruding into the vena renalis. The tumor was diagnosed as a RCC.

CLAIMS

1. Monoclonal antibody specific to an antigen of renal cell carcinoma selected from the group consisting of antigen G 250, antigen RC 38, antigen RC 3, antigen RC 69, and antigen RC 154.

2. An antigen binding fragment of the monoclonal antibody of Claim 1.

3. A composition for immunoscintigraphy of renal cell carcinoma, comprising a radiolabeled monoclonal antibody specific for an antigen selected from the group consisting of antigen G 250, antigen RC 3, antigen RC 38, antigen RC 69, and antigen 154.

4. The composition of Claim 3, wherein the antibody is specific for antigen G 250 or RC 38.

5. A composition of Claim 3, wherein the antibody is radiolabeled with a radioisotope selected from the group consisting of $^{123}$Iodine, $^{125}$Iodine, $^{131}$Iodine, $^{99m}$Technetium or $^{111}$Indium.

6. A composition for immunoscintigraphy of RCC, comprising a radiolabeled antigen binding fragment of a monoclonal antibody specific for

an antigen selected from the group consisting of antigen G 250, antigen RC 3, antigen RC 38, antigen RC 69, and antigen RC 154.

7. The composition for immunoscintigraphy of RCC of Claim 6 wherein the antigen binding fragment is an $F(ab')_2$ or Fab' fragment.

8. A composition of Claim 6, wherein the antibody is specific for antigen G 250 or RC 38.

9. A composition of Claim 6, wherein the antibody or fragment is radiolabeled with a radioisotope selected from the group consisting of $^{123}$Iodine, $^{125}$Iodine, $^{131}$Iodine, $^{99m}$Technetium or $^{111}$Indium.

10. A composition for immunoscintigraphy of RCC, comprising a mixture of radiolabeled anti-RCC monoclonal antibodies or antigen binding fragments thereof, the monoclonal antibodies being antibodies specific for antigens selected from the group consisting of antigen G 250, antigen RC 38, antigen RC 3, antigen RC 69, and antigen RC 154.

11. A composition of Claim 10, wherein the fragments are $F(ab')_2$ or Fab' fragments.

12. A composition of Claim 10, wherein the mixture comprises antibody specific for antigen G 250 and antibody specific for RC 38.

13. A composition of Claim 10, wherein the mixture comprises an $F(ab')_2$ or Fab' fragment of antibody specific for RC38 and an $F(ab')_2$ or Fab' fragment of antibody specific for G250.

14. A composition of Claim 10, wherein the antibody is radiolabeled with a radioisotope selected from the group consisting of $^{123}$Iodine, $^{125}$Iodine, $^{131}$Iodine, $^{99m}$Technetium or $^{111}$Indium.

15. A method for detecting and localizing renal cell carcinoma (RCC), comprising the steps of:

a) injecting a human subject parenterally with an antibody or antibody fragment specific for an antigen of RCC selected from the group consisting of antigen G 250, antigen RC 38, antigen RC 3, antigen RC 69, and antigen RC 154, the antibody being labelled with a signal generating label;

b) allowing sufficient time for the labeled antibody to accumulate at the site of the RCC; and

c) detecting the signal with a signal detecting means; and

d) converting the detected signal to an image of the RCC.

16. A method of Claim 15, wherein the antibody fragment is an $F(ab')_2$ fragment or a Fab' fragment.

17. A method of Claim 15, wherein the antibody or fragment thereof is specific for antigen G 250 or RC 38.

18. A method of Claim 15, wherein a mixture of antibody against RC 38 and G 250 or fragments thereof is injected.

19. A method of Claim 15, wherein the antibody is radiolabeled with a radioisotope selected from the group consisting of $^{123}$Iodine, $^{125}$Iodine, $^{131}$Iodine, $^{99m}$Technetium or $^{111}$Indium.

20. A method of Claim 18, wherein the signal detecting means is a gamma camera.

21. A method for determining the presence of RCC in a tissue sample, comprising the steps of:

a. contacting a tissue specimen from a patient suspected of having RCC with an antibody specific for an antigen selected from the group consisting of G 250, RC 38, RC 3, RC 69 and RC 154 under conditions which permit binding of the antibody to cells in the sample bearing the antigen; and

b. determining whether the antibody binds to cells by immunohistochemical techniques, the binding of the antibody being an indicator of the presence of RCC.

22. A method of Claim 21 wherein the antibody is specific for antigen G 250 or RC 38.

23. A method of immunotherapy of RCC, comprising administering an anti-tumor amount of antibody specific for the RCC antigens selected from the group consisting of G 250, RC 38, RC 3, RC 69 and RC 154.

24. A method of Claim 23, wherein the antibody is conjugated to an anti-cancer agent.

25. A method of Claim 23, wherein a mixture of antibodies is administered.

26. A method of Claim 23, wherein the RCC antigens are selected from G 250 and RC 38.

27. A method of Claim 23, wherein the antibody is of the IgE class.

28. An antibody for targeting a cytotoxic cell to RCC, having dual specificity, a first specificity for an RCC antigen selected from the

group consisting of RC 38, RC 4 and G250; and a second specificity for a human cytotoxic cell.

29. An antibody of Claim 28, wherein the human cytotoxic cell is a monocyte, a cytotoxic T cell or an NK cell.

30. An antibody having a dual specificity, a first specificity for the T3 antigen of T cells and a second specificity for an RCC antigen selected from the group consisting of RC 4, RC 38 and G250.

31. Monoclonal antibody RC 38.

32. Monoclonal antibody G 250.

33. Monoclonal antibody specific for G 250 or RC 38 of the IgE class.

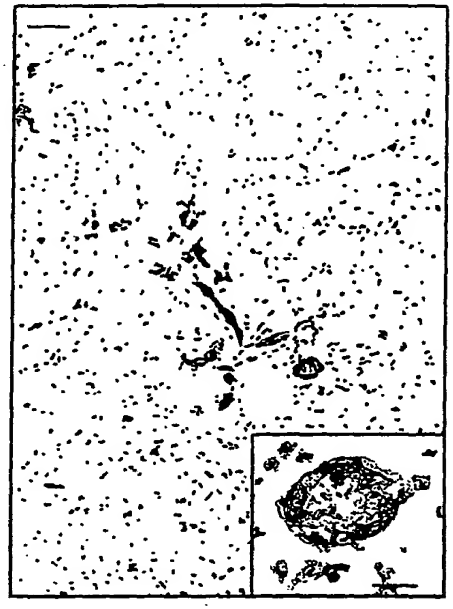


FIGURE 1

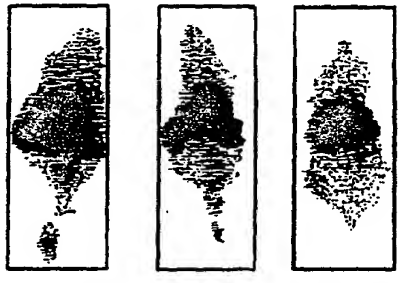
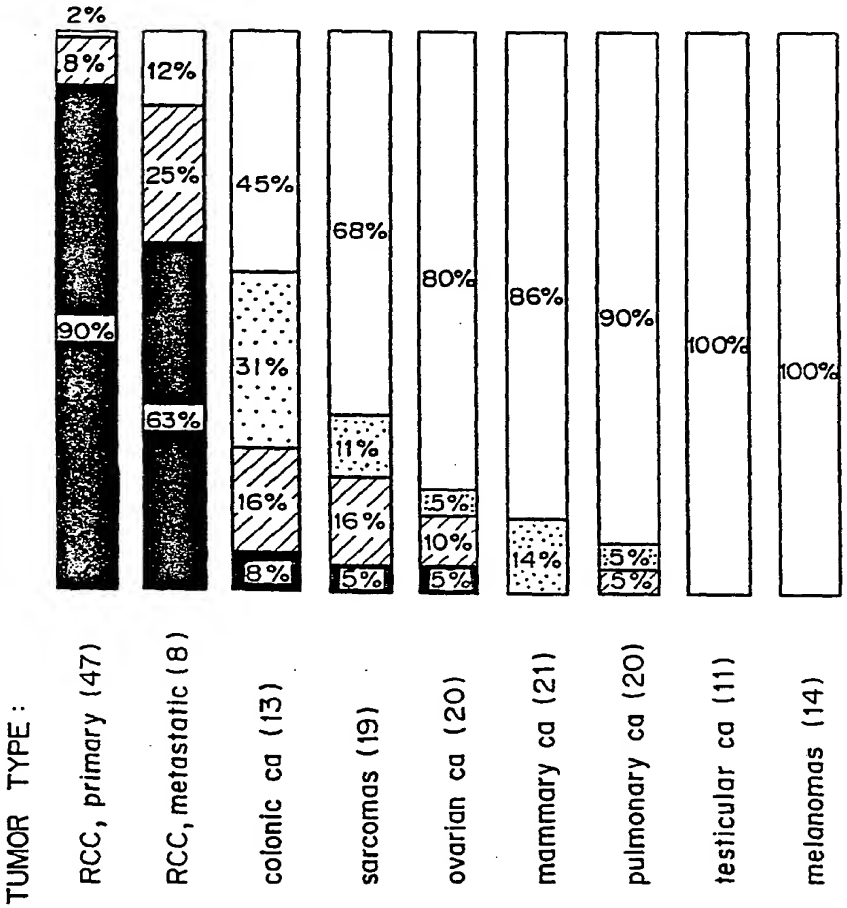


FIGURE 3a FIGURE 3b FIGURE 3c

FIGURE 2a

FIGURE 2b




Reactivity Pattern of Mab G 250

*FIGURE 4*

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/01511

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC C 07 K 15/00;
IPC[4]: A 61 K 49/02; G 01 N 33/574; A 61 K 39/395; C 12 P 21/00;
// C 12 N 15/00; C 12 N 5/00; [C 12 P 21/00; C 12 R 1:91]

## II. FIELDS SEARCHED

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| IPC[4] | A 61 K; C 12 P |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

| Category [10] | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No. [13] |
|---|---|---|
| X | Biological Abstracts, vol. 83, 1987 (Philadelphia, PA, US), E. Oosterwijk et al.: "Monoclonal antibody G-250 recognizes a determinant present in renal-cell carcinoma and absent from normal kidney", abstract no. 25749, & Int J Cancer 38(4): 489-494, 1986, see the whole abstract | 1-14,21,22, 28-30,32,33 |
| X | Chemical Abstracts, vol. 105, 1986 (Columbus, Ohio, US), S.J. Luner et al.: "Monoclonal antibodies to kidney and tumor-associated surface antigens of human renal cell carcinoma", see page 462, abstract no. 207328v, & Cancer Res. 1986, 46(11); 5816-20 | 1-14,21,22, 28-33 |
| X | EP, A, 0210970 (REGENTS OF THE UNIVERSITY OF MINNESOTA) 4 February 1987, see column 3, line 18 - column 4, line 17; column 23, lines 15-37; claims 1-5, 9-14 | 1-14,21,22, 28-33 |

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 15th September 1988 | - 7. 10. 88 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUROPEAN PATENT OFFICE | P.C.G. VAN DER PUTTEN |

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No |
|---|---|---|
| X | Chemical Abstracts, vol. 104, 1986 (Columbus, Ohio, US), R.L. Vessella et al.: "Monoclonal antibodies to human renal cell carcinoma: recognition of shared and restricted tissue antigens", see page 385, abstract no. 18436x, & Cancer Res. 1985, 45(12, Pt. 1), 6131-9 | 1-14,21,22, 28-33 |
| X | Biological Abstracts, vol. 80, 1985 (Philadelphia, PA, US), T.D. Moon et al.: "A highly restricted antigen for renal cell carcinoma defined by a monoclonal antibody", abstract no. 51329, & Hybridoma 4(2): 163-172, 1985, see the whole abstract | 1-14,21,22, 28-33 |
| X | EP, A, 0160250 (SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH) 6 November 1985, see page 7, lines 13-19; page 26, line 17 - page 27, line 5; page 31, lines 12-16 | 1-14,21,22, 28-33 |
| A | EP, A, 0119528 (SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH) 26 September 1984, see page 7, lines 7-15; page 14, line 18 - page 15, line 7; page 16, lines 24-30; claims 1-9 | 1-14,21,22, 28-33 |

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET PCT/ISA/210 (2)

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers XX, because they relate to subject matter not required to be searched by this Authority, namely:

xx claim numbers 15-20, 23-27
see PCT - Rule s9.1 (iv) :
Method for treatment of the human or animal body by therapy, diagnostic method practised on the human or animal body

2. [ ] Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8801511 SA 22953

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/09/88
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP-A- 0210970 | 04-02-87 | AU-A- 6032786 | 29-01-87 |
| | | JP-A- 63036794 | 17-02-88 |
| EP-A- 0160250 | 06-11-85 | JP-A- 61022028 | 30-01-86 |
| | | US-A- 4713352 | 15-12-87 |
| EP-A- 0119528 | 26-09-84 | JP-A- 59219299 | 10-12-84 |
| | | CA-A- 1221648 | 12-05-87 |